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A Rapid, Quantitative Bioassay for Detecting Phytotoxic Gases Using Stress-Ethylene

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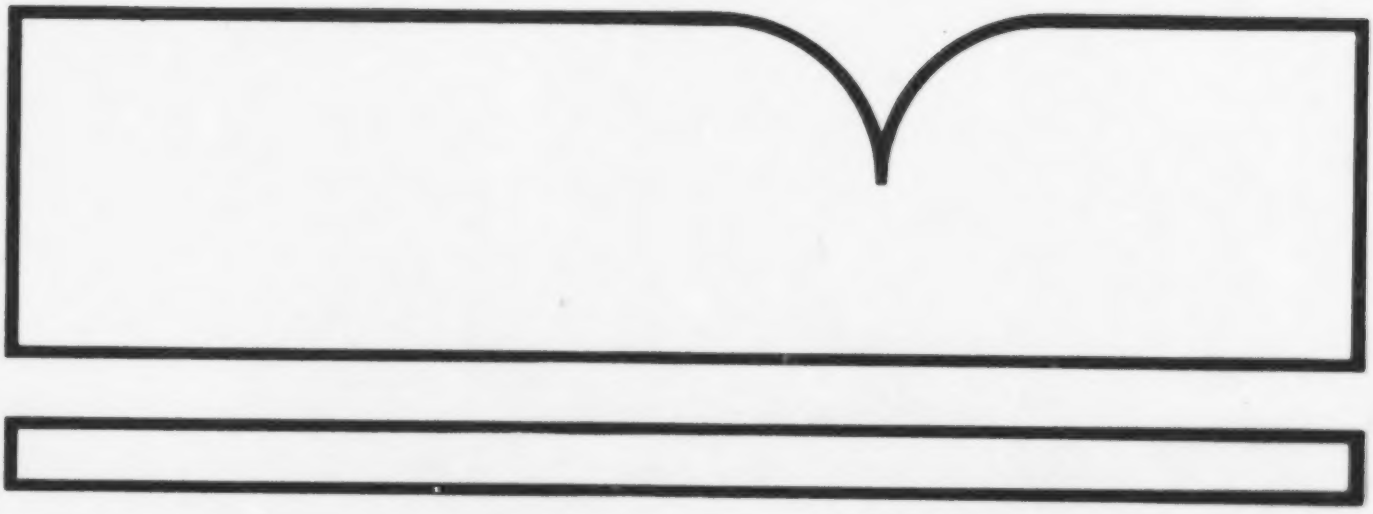

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PRELIMINARY COMMUNICATION

A RAPID, QUANTITATIVE BIOASSAY FOR DETECTING PHYTOTOXIC GASES USING STRESS-ETHYLENE

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Abstract. A simple bioassay for detecting phytotoxic air pollutants has been developed. Wheat (*Triticum aestivum* L.) seedlings growing on agar medium in test-tubes are exposed to pollutants for 2 h . Stress-ethylene produced by the seedlings during exposure to the pollutants is collected in the test-tube and quantitatively measured by gas chromatography.

Although a number of gaseous substances may represent a phytotoxic hazard, only a few have been tested, studied and regulated to prevent aesthetic and economic losses before release into the atmosphere (Heggestad, 1968; Hindawi, 1970; and Jacobson and Hill, 1970). The primary method of detecting phytotoxicants in air samples has been to observe tissue injury on plants exposed to pollutants (Hindawi, 1970; Jacobson and Hill, 1970; and LaCasse and Treshow, 1978). However, this and other air pollution screening bioassays (Todd and Probst, 1963; Koukol and Dugger, 1967; Runeckles and Resh, 1975; Tingey *et al.*, 1976 and 1977; Knudsen *et al.*, 1977) have involved large commitments of time and facilities because of relatively long-term growth periods required for test plants plus large quantities of pollutant and special fumigation chambers necessary for exposing plants to pollutants. In order to establish safe release guidelines, government agencies and industrial laboratories need a rapid, simple and accurate means of screening smokestack emissions, industrial compounds, and other air samples for phytotoxicants.

This study reports the use of a rapid, quantitative bioassay for detecting phytotoxic activity of air pollutants using pollutant-induced stress-ethylene production from wheat seedlings growing in test-tubes.

Wheat, *Triticum aestivum* L., cv. Olaf, seeds were cold stratified for 5 days at 5°, surface sterilized in a 1% sodium hypochlorite solution for 10 minutes, rinsed with distilled water, and planted in 23 ml test-tubes (1.5 x 15 cm) containing 3 ml of sterilized agar growth medium [1% agar and half-strength Hoagland's solution (Hoagland and Arnon, 1950)]. The test-tubes containing seeds were stoppered with cotton plugs to prevent airborne pathogenic contamination and placed into controlled environment chambers for germination and growth [16 h day/8 h night cycle; 65 microeinsteins m^{-2} s^{-1} (400-700 nm); with a constant temperature of 18°]. To provide for water loss and to maintain stomatal openings, 0.5 ml of half-strength Hoagland's solution was supplied to plants the day before exposure to pollutants.

At 6 days after planting, the cotton plugs of each test-tube were replaced with a rubber serum cap and a predetermined amount of pollutant injected into each sealed tube with a syringe and needle to give a known initial pollutant concentration within each test-tube. The tubes were then returned to the controlled environment chamber used in growing the seedlings for a treatment period of 2 h during which the pollutant interacted with the seedling and the stress-ethylene produced by the seedlings in response to the pollutant treatment was trapped in the sealed test-tube. A 2 ml gas sample was removed from each test-tube at the end of the treatment period and quantitatively analyzed for ethylene by gas chromatography (system sensitive to 5 ppb C_2H_4) (Craker, 1971). Following ethylene analysis, fresh weight

of top growth from each plant was determined.

Control seedlings were grown and treated in the same manner as pollutant-treated seedlings, except no pollutant was added to test-tubes containing plant material. Differences in the amounts of ethylene produced per unit fresh weight of tissue by pollutant-treated plants and control plants were used to indicate the stress-ethylene resulting from pollutant exposures. All tests contained a minimum of 5 samples per treatment and were replicated a minimum of 3 times.

Treatment of the wheat seedlings with known phytotoxic air pollutants produced measurable increases in stress-ethylene within 2 h (Table 1). Treatment with carbon monoxide [a non-phytotoxic gas except at very high concentrations (LaCasse and Treshow, 1978)] did not stimulate ethylene production above that of control plants not exposed to pollutants.

Table 1. Production of Ethylene from Wheat Seedlings Treated With Air Pollutants

Pollutant	Pollutant Concentration[1] ($mg/m^3 \cdot 10^3$)	Ethylene Production[2] ($n\ell/g$ Fresh wt·2 hr)
NO_2	36.8	2.69 ± 0.22
H_2S	27.1	2.10 ± 0.18
SO_2	20.4	2.22 ± 0.22
Cl_2	17.0	4.18 ± 0.43
Control	0	1.46 ± 0.16

1. Initial concentration inside test-tube. The actual concentra- or dose with which plants are treated may differ due to absorption of pollutant by agar growth media and test-tube walls.
2. Means ± S.E.

The relationship between stress-ethylene production and treatment of seedlings with different amounts of SO_2 is illustrated in Figure 1. For all the phytotoxic gases tested, there was a range of pollutant concentrations where an increase in concentration initiated a linear increase in ethylene production (Data not shown).

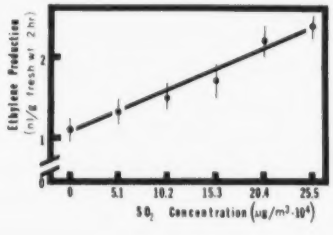

Figure 1. Stress-ethylene production by wheat seedlings exposed to SO_2. Vertical lines are standard error of means.

Results from this study indicated that stress-ethylene production by wheat seedlings exposed to pollutants could be used as a rapid and quantitative test for the presence of phytotoxicants in air samples. The developed technique is similar to that reported in other studies where stress-ethylene produced by plants fumigated with ozone (Tingey *et al.*, 1976),

sulfur dioxide (Bressan *et al.*, 1978), and chlorine (Tingey *et al.*, 1977) has been used to measure and quantify air pollution effects on plants; except in this instance, young seedlings growing in test-tubes were used as the test plant material and the test-tubes served both the exposure chamber and container for collection of stress-ethylene.

The amount of ethylene produced by pollutant-treated wheat seedlings was related to the amount of the pollutant to which the plants were exposed. With each pollutant, ethylene production increased as the concentration of the pollutant used in treating the plants was increased. This relationship between ethylene production and pollutant concentration is similar to the results of other investigations where exposure to increased concentrations of pollutants increased ethylene production in plants (Craker, 1971; Tingey *et al.*, 1976 and 1977; and Bressan *et al.*, 1978).

Although the bioassay has reduced the need for greenhouse and fumigation facilities as well as minimized the quantities of test gases necessary for a phytotoxicity test, applications are currently limited to situations where air samples contain large amounts of pollutants. A positive indication of phytotoxicity with SO_2, Cl_2, NO_x, and H_2S required treatment with pollutant concentrations up to 20 times those normally shown to injure plants in ambient conditions (LaCasse and Treshow, 1978). The exact reasons for low sensitivity of the bioassay are unknown, but an important factor may be the rapid degradation of the pollutant in the static environment of the sealed test-tube used in treating plants. For example, under conditions approximating those of the bioassay, the rate for SO_2 absorption into water is about $10^6 s^{-1}$ (Carmichael and Peters, 1979; Möller, 1980) and the equilibrium constant is about 10^{-2}M (Erickson *et al.*, 1977). Any SO_2 added to the environment of the sealed tube of the bioassay would undoubtedly dissolve into the water of the agar within minutes and be unavailable for inducing stress-ethylene production from plant leaves.

The bioassay outlined in this report should aid in the detection and quantification of phytotoxic pollutants in air samples containing high pollutant concentrations. Since the test plants are grown on an agar medium in test-tubes, the procedure can be replicated a large number of times in a matter of hours, requiring only a small amount of air pollutant and a minimum amount of space. The measurement of stress-ethylene production provides an unbiased estimate of phytotoxic activity.

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